Proposed New Claims (Serial No. 10/062,308)

47. A computer-implemented method for determining if a biomarker is present within a cellular compartment in a tissue section, comprising:

incubating the tissue section with an appropriate solution containing a first stain that specifically labels the biomarker and an appropriate solution containing a second stain that specifically labels the cellular compartment, thereby obtaining a tissue section that contains a first stain labeled biomarker and a second stain labeled cellular compartment;

obtaining a first high resolution image of the tissue section using an upright or inverted optical microscope set at a wavelength appropriate to detect the first stain, wherein the image comprises multiple pixel locations and each of the multiple pixel locations is analyzed to identify pixel locations having an intensity value indicative of the presence of the first stain labeled biomarker;

obtaining a second high resolution image of the tissue section using an upright or inverted optical microscope set at a wavelength appropriate to detect the second stain, wherein the image comprises multiple pixel locations and each of the multiple pixel locations is analyzed to identify pixel locations having an intensity value indicative of the presence of the second stain labeled cellular compartment; and

analyzing corresponding pixel locations in the first and second images to identify pixel locations that have both an intensity value indicative of the presence of the first stain in the first image and an intensity value indicative of the presence of the second stain in the second image, so as to thereby determine the presence of biomarker in the cellular compartment.

48. A method of claim 47, wherein the amount of the biomarker present within the cellular compartment is determined by summing the intensity values of the pixels

associated with the first stain within the cellular compartment and dividing the sum by the number of pixels associated with the cellular compartment.

49. The method of claim 47, wherein the cellular compartment is selected from the group consisting of a cell nucleus, a cytoplasm, a nuclear membrane, a cellular membrane, a mitochondria, an endoplasmic reticulum, a peroxisome, a lysosome or a user defined area.

50. The method of claim 47, wherein the tissue has a thickness of about five microns.

51. The method of claim 47, wherein the high resolution image comprises 1024 x 1024 pixel locations.

52. The method of claim 47, wherein the biomarker is selected from the group consisting of a protein, a peptide, a nucleic acid, a lipid or a carbohydrate.

53. The method of claim 47, wherein the tissue section is fixed.

54. The method of claim 47, wherein the first and second stains are fluorescent stains.

55. The method of claim 54, wherein the fluorophore of the fluorescent stain is 4',6-diamidino-2-phenylindole (DAPI), Cy3 or Cy-5-tyramide.

56. The method of claim 47, wherein the biomarker is selected from the group consisting of: Her-2/neu, estrogen receptor, progesterone receptor and epidermal growth factor receptor.

57. The method of claim 47, wherein, out-of-focus information is reduced from the first and second high resolution images.

58. The method of claim 47, wherein a mask is applied to the first and second high resolution images.

59. A computer-implemented method for determining if a biomarker is present within a first or a second cellular compartment in a tissue section, comprising:

incubating the tissue section with an appropriate solution containing a first stain that specifically labels the biomarker, an appropriate solution containing a second stain that specifically labels the first cellular compartment and an appropriate solution containing a third stain that specifically labels the second cellular compartment, thereby obtaining a tissue section that contains a first stain labeled biomarker, a second stain labeled first cellular compartment and a third stain labeled second cellular compartment;

obtaining a first high resolution image of the tissue section at a wavelength appropriate to detect the first stain, wherein the image comprises multiple pixel locations and each of the multiple pixel locations is analyzed to identify pixel locations having an intensity value indicative of the presence of the first stain labeled biomarker;

obtaining a second high resolution image of the tissue section at a wavelength appropriate to detect the second stain, wherein the image comprises multiple pixel locations and each of the multiple pixel locations is analyzed to identify pixel locations having an intensity value indicative of the presence of the second stain labeled cellular compartment;

obtaining a third high resolution image of the tissue at a wavelength appropriate to detect the third stain, wherein the image comprises multiple pixel locations and each of the multiple pixel locations is analyzed to identify pixel locations having an intensity value indicative of the presence of the third stain labeled second cellular compartment; and

analyzing corresponding pixel locations in the three images to identify pixel locations that have an intensity value indicative of the presence of the first stain in the first image and an intensity value indicative of the presence of the second stain in the second image, so as to thereby determine the presence of biomarker in the first cellular compartment and pixel locations that have an intensity value indicative of the presence of the first stain in the first image and an intensity value

indicative of the third stain in the third image, so as to thereby determine the presence of the biomarker in the second cellular compartment.

60. A method of claim 59, wherein the amount of the biomarker present within the cellular compartment is determined by summing the intensity values of the pixels associated with the first stain within the cellular compartment and dividing the sum by the number of pixels associated with the cellular compartment.

61. The method of claim 59, wherein the cellular compartment is selected from the group consisting of a cell nucleus, a cytoplasm, a nuclear membrane, a cellular membrane, a mitochondria, an endoplasmic reticulum, a peroxisome, a lysosome or a user defined area.

62. The method of claim 59, wherein the tissue has a thickness of about five microns.

63. The method of claim 59, wherein the high resolution image comprises 1024 x 1024 pixel locations.

64. The method of claim 59, wherein the biomarker is selected from the group consisting of a protein, a peptide, a nucleic acid, a lipid or a carbohydrate.

65. The method of claim 59, wherein the tissue section is fixed.

66. The method of claim 59, wherein the first and second stains are fluorescent stains.

67. The method of claim 66, wherein the fluorophore of the fluorescent stain is 4',6-diamidino-2-phenylindole (DAPI), Cy3 or Cy-5-tyramide.

68. The method of claim 59, wherein the biomarker is selected from the group consisting of: Her-2/neu, estrogen receptor, progesterone receptor and epidermal growth factor receptor.

69. The method of claim 59, wherein the second stain reacts with a marker that is selected from the group consisting of: cytokeratin, beta catenin, alpha catenin and vimentin.